

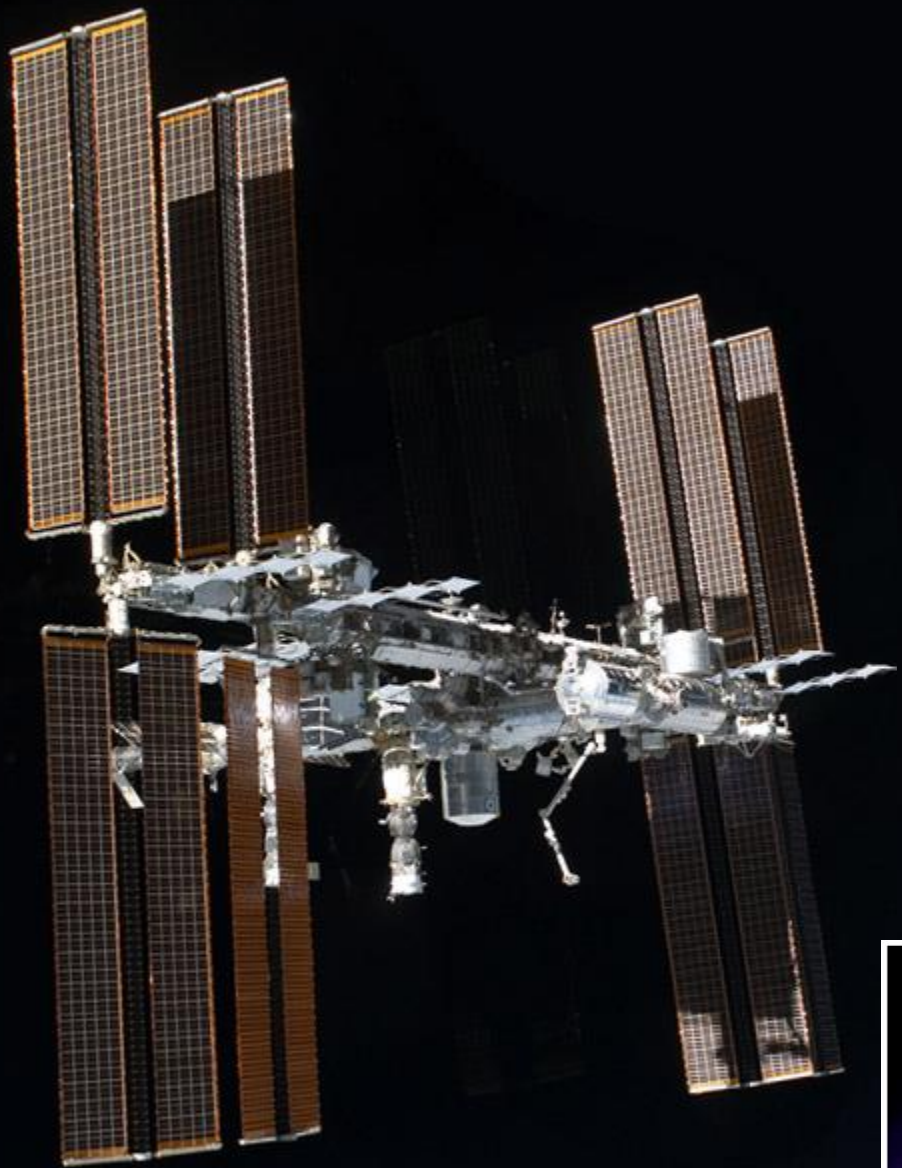
Novel End-to-End Molecular Biology Approach for Direct Nanopore 1D cDNA Sequencing of Reverse Transcribed mRNAs Purified from Cell Cultures by the NASA ISS WetLab2 SPM.

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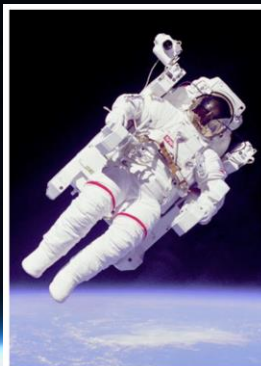


U.S. NATIONAL LABORATORY



POWERED THROUGH PARTNERSHIP

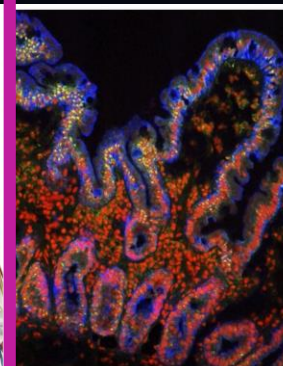
Organism



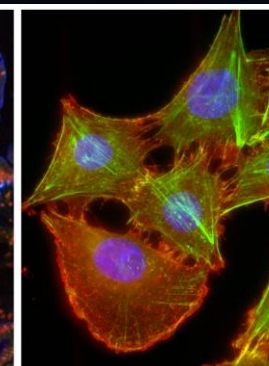
Systems



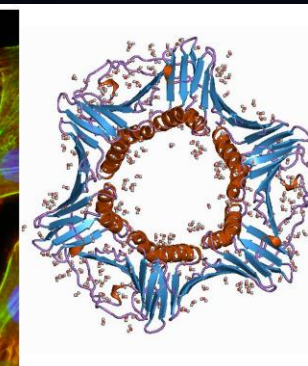
Tissues



Cells



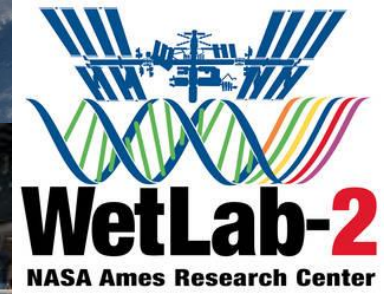
Molecules



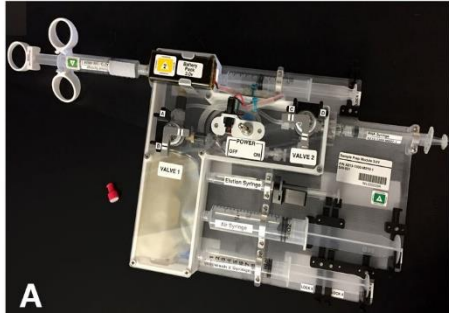
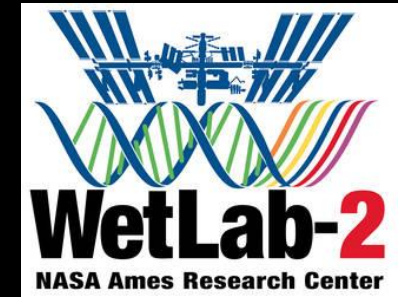
What has been done on-board the ISS National Lab



- g-DNA/total-RNA isolation from eukaryote, prokaryote and viral samples (WL2 – SpaceX-8) and PCR analysis tech demo.
- Real time sequencing with the MinION (Bio-Seq) from pre-prepared library.



WetLab-2 Platform Components



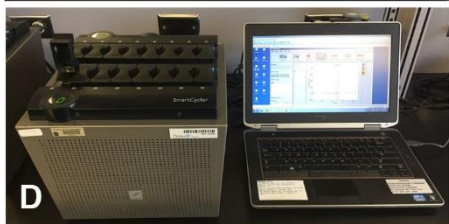
Sample Preparation Module (SPM)
Nucleic Acid Purification (RNA, DNA)
on ISS from Biological Samples
(Microbial, Tissue, Blood etc.)
Flight Validated on ISS
(Room Temperature Storage)



Fluid Transfer, Debubbling and
Centrifugation Tools
Flight Validated on ISS

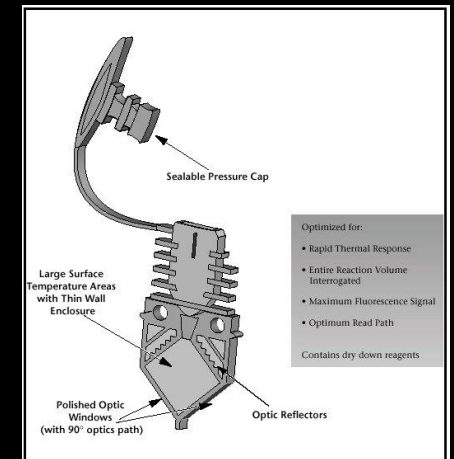


Lyophilized All-In-One Reverse
Transcription of mRNAs and PCR
Amplification of cDNAs or gDNA
Flight Validated on ISS
(Room Temperature Storage)

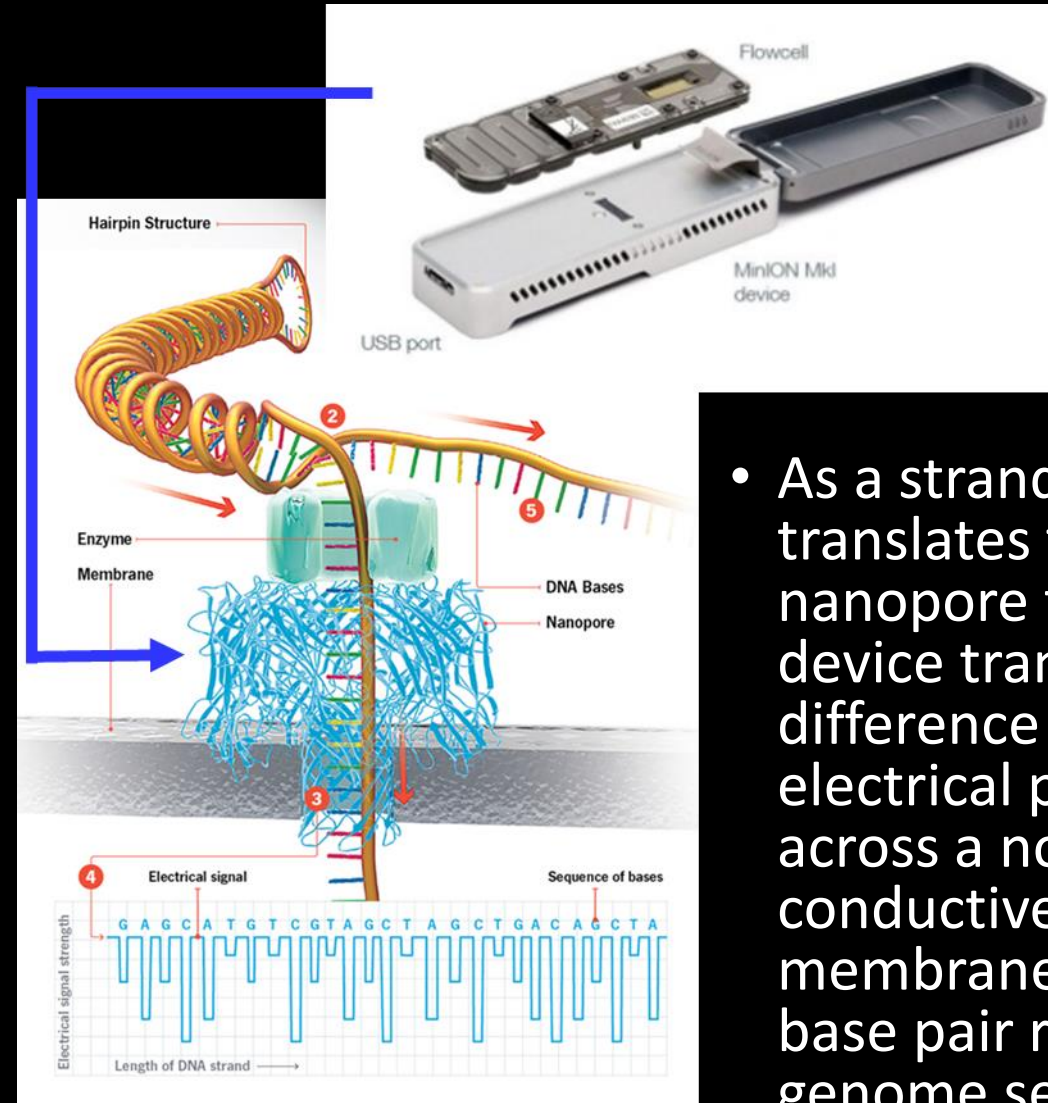
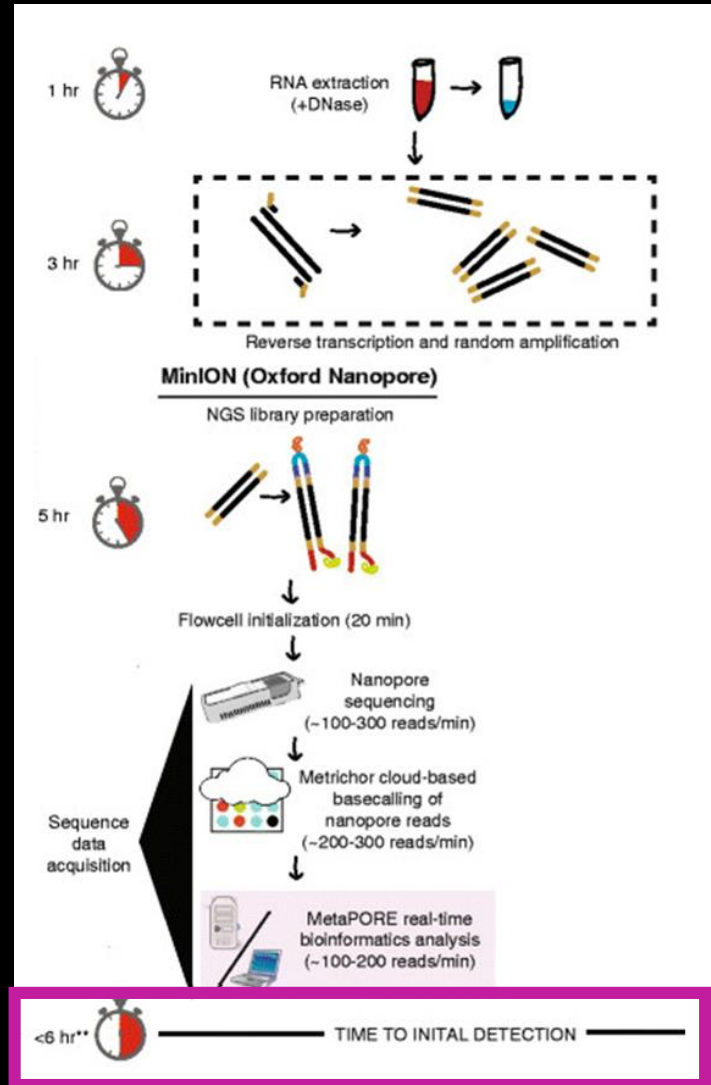


RT PCR Thermal Cycler
for Reverse Transcription and PCR
Amplification of mRNAs or gDNAs
Flight Validated on ISS

- Sample Preparation Module (SPM)
 - Nucleic acid purification (RNA, DNA) on ISS from biological samples.
 - (Microbial, Tissue, Cells, Blood, etc.)
- Cyphid Thermal Cycler (Smartcycler)
 - Controlled thermal cycling
 - 16 sample capacity
 - 16 independently controlled ports.



MinION Biomolecule Sequencer

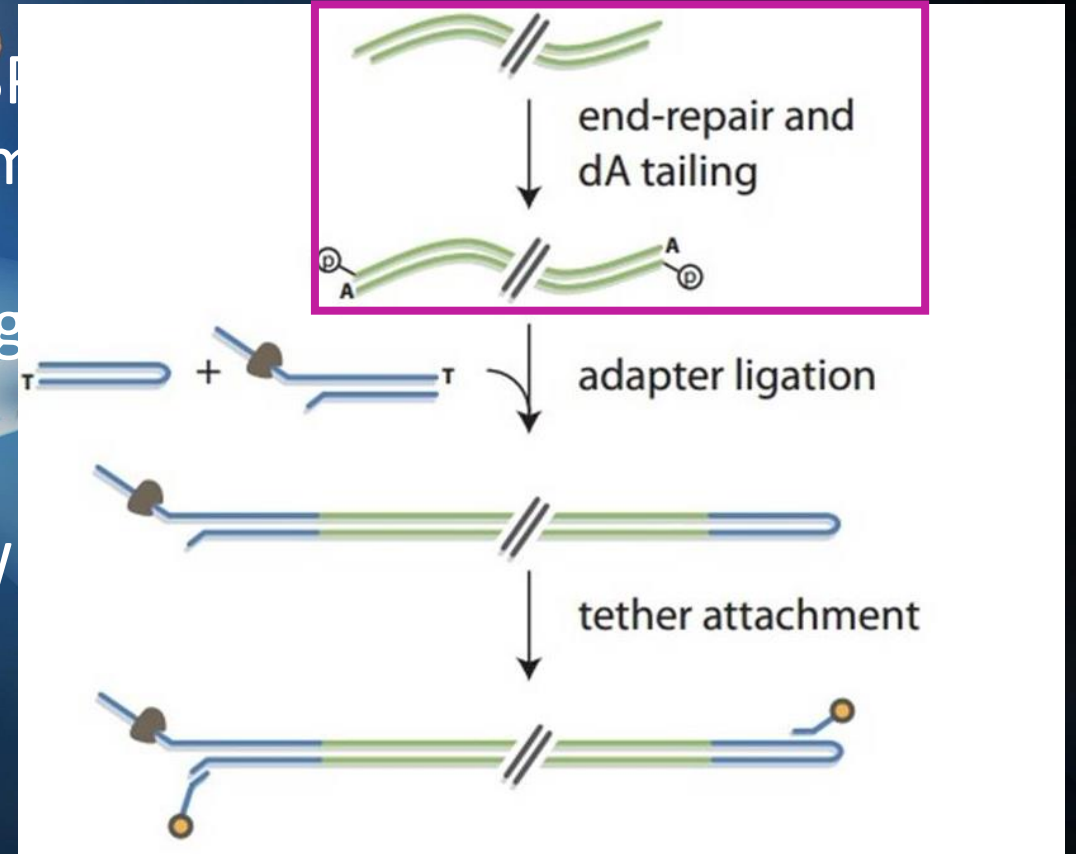


- As a strand of DNA translates the nanopore the MinION device translates the difference in electrical potentials across a non-conductive membrane into single base pair resolution genome sequences.

Greninger, Alexander L., et al. "Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis." *Genome Medicine* 7 (2015).

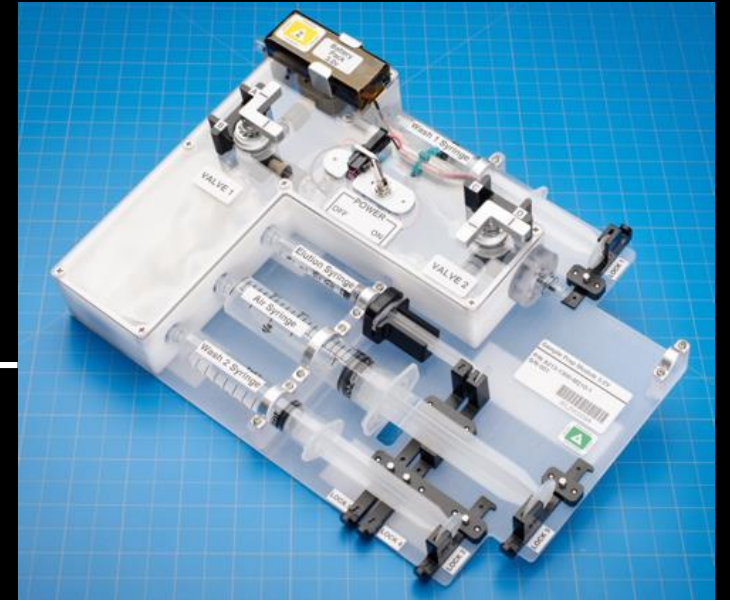
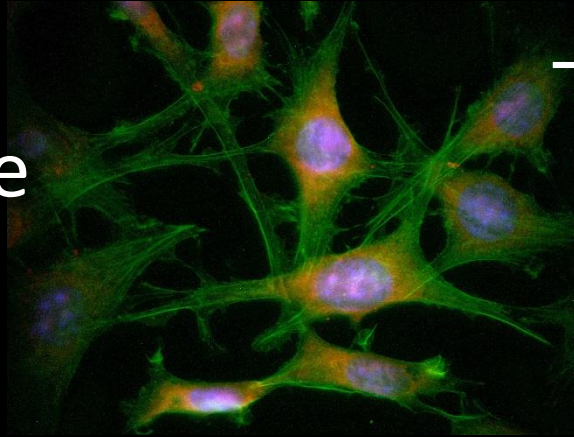
End-to-End Methodology

- Utilize the Sample Preparation Module (SPM) to process a given sample of tissue, cells, or microbiome
- Purify the mRNA from the total RNA using an oligo-dT isolate sample with poly-A tailing.
- Synthesize cDNA and amplify using the WTA
- Generate pre-sequencing library
 - General 3 step system
- Purify sample and load into the MinION for sequencing and live basecalling

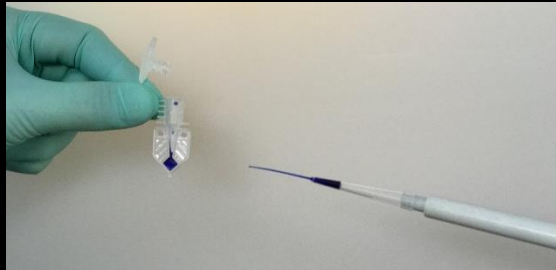


Proof of Concept Experiment: End-to-End Library Isolation and Purification

- Sample Preparation Module (SPM) Total RNA Isolation from MLOY4 mouse osteocyte-like cell colonies.



- Transfer tube



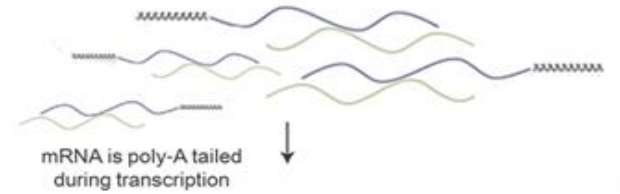
- Magnetic Bead Clean-Up mRNA preferential binding



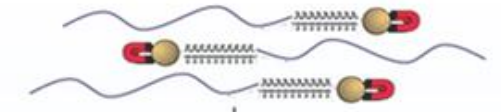
POC: End-to-End pre-sequencing library prep

- Our protocol takes advantage of mRNA's native poly(A) tail to eliminate end-prep for adapter ligation.
- After magnetic bead cleanup
 1. Synthesize 1st and 2nd strand cDNA using SuperScript II and WL2 SmartCycler for thermal cycling
 2. Attach Tether and Adapter via ligation to the polyA and polyT endcaps respectively.

Isolation of total RNA, collection from SPM WL2



mRNA separation with magnetic Dynabeads



Initiate reverse transcription at poly-A tail



cDNA 1st strand synthesis



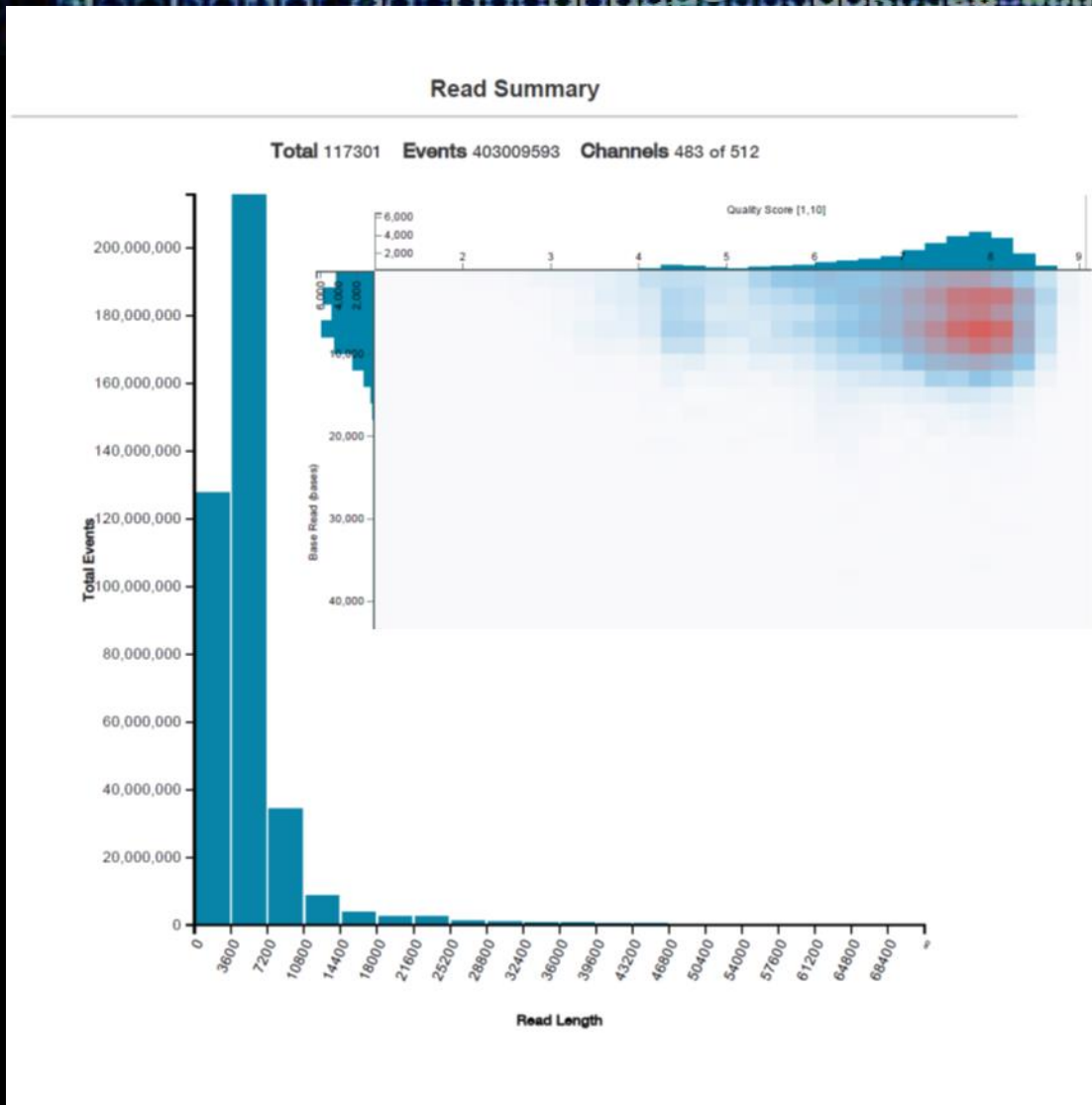
cDNA 2nd strand synthesis



Adapter Ligation



POC: End-to-End Sequencing Run MinION 6 hour with Local Basecalling Enabled



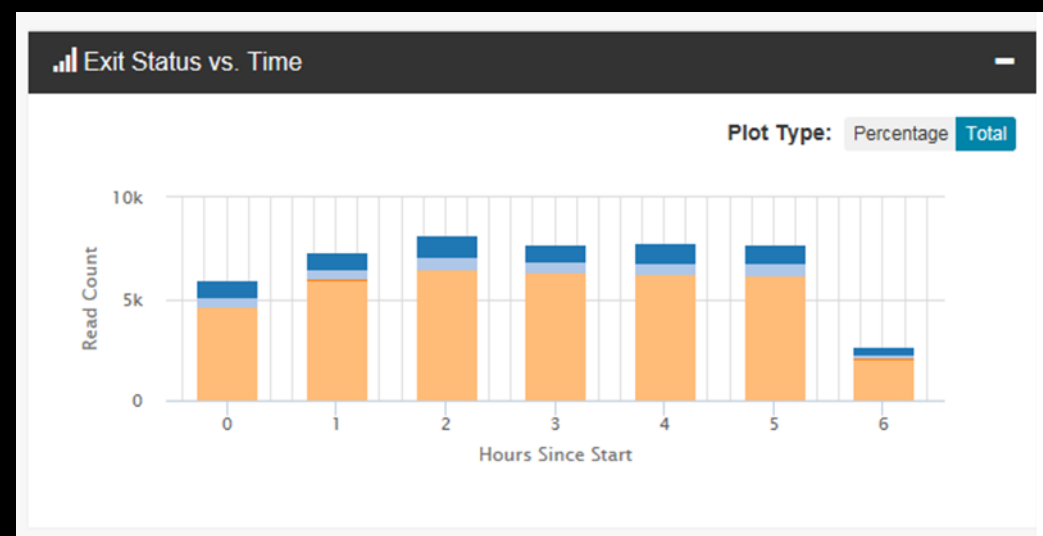
- The MinION device translates the difference in electrical potentials across a non-conductive membrane into single base pair resolution genome sequences.
- MinKNOWN, the MinION software, identifies sequence lengths and basecalling reads in real time.

POC: Metrichor Analysis of Sequencing run Success

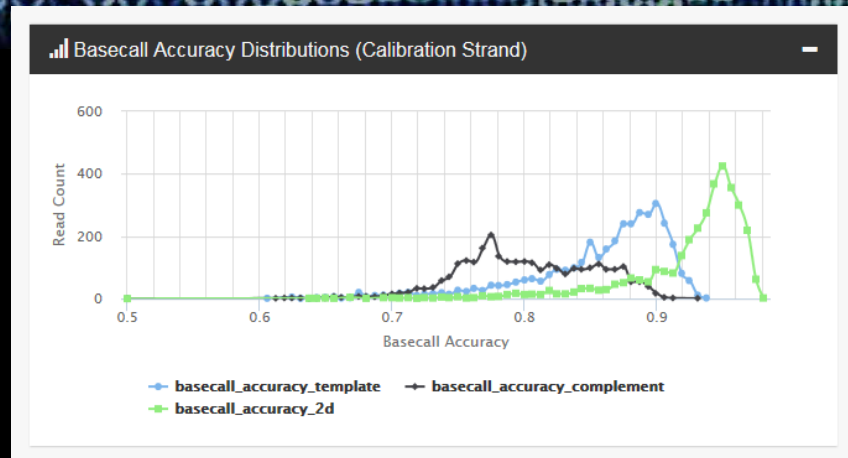
Basecalling 2D		
Read Count per Run ID		
Run ID	Read Count	
642da14a5ae835391d2c62	47071	<div><div></div></div> Showing ✓
2c346ad8344b50303d497	90	<div><div></div></div> Showing ✓

Read Count per Exit Status			
Exit Status	Read Count		
Workflow successful	38086	<div><div></div></div>	Showing ✓
2D basecall could not be performed	5786	<div><div></div></div>	Showing ✓
2D basecall failed quality filters	3254	<div><div></div></div>	Showing ✓
Exception thrown	35	<div><div></div></div>	Showing ✓

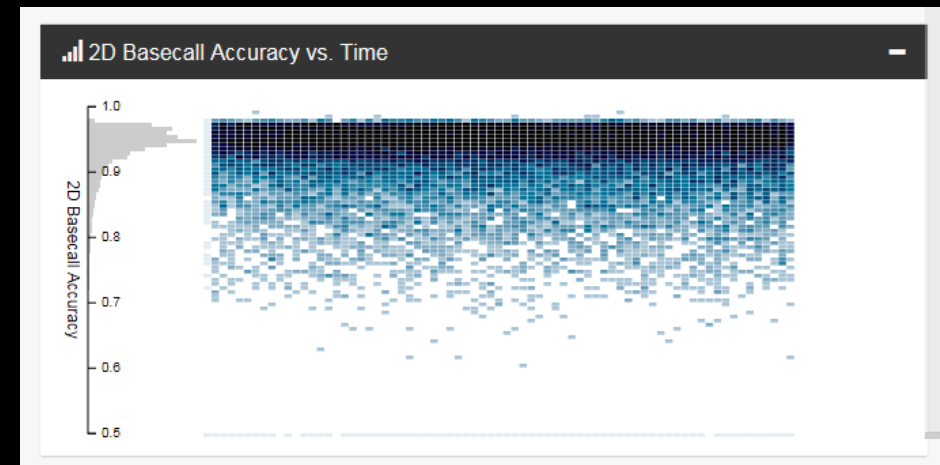
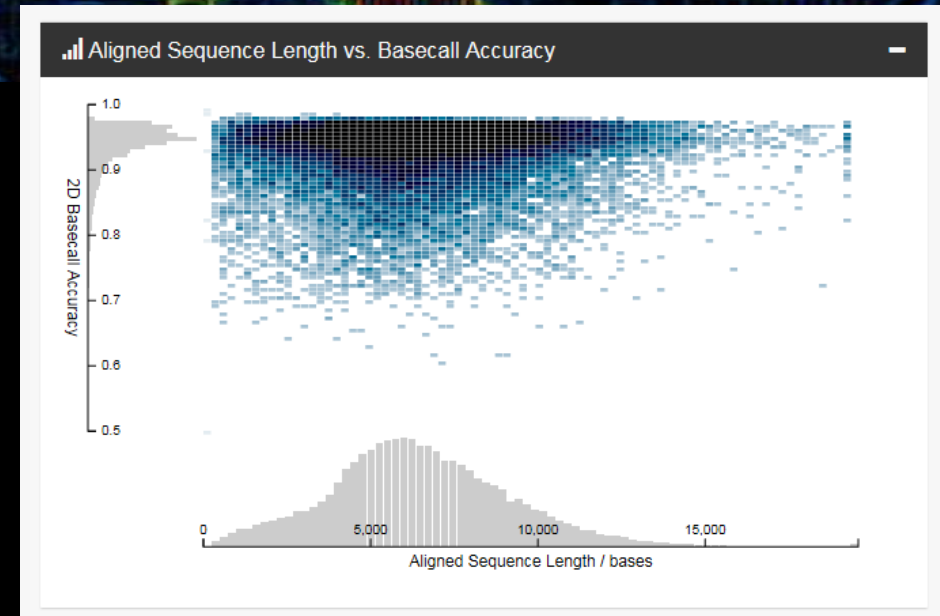
- After basecalling data was uploaded into Metrichor (a cloud based analysis program) and evaluated for
 - Successful basecall
 - Exit Status – associated with successful basecalling



POC: Metrichor Analysis of Sequencing run Success

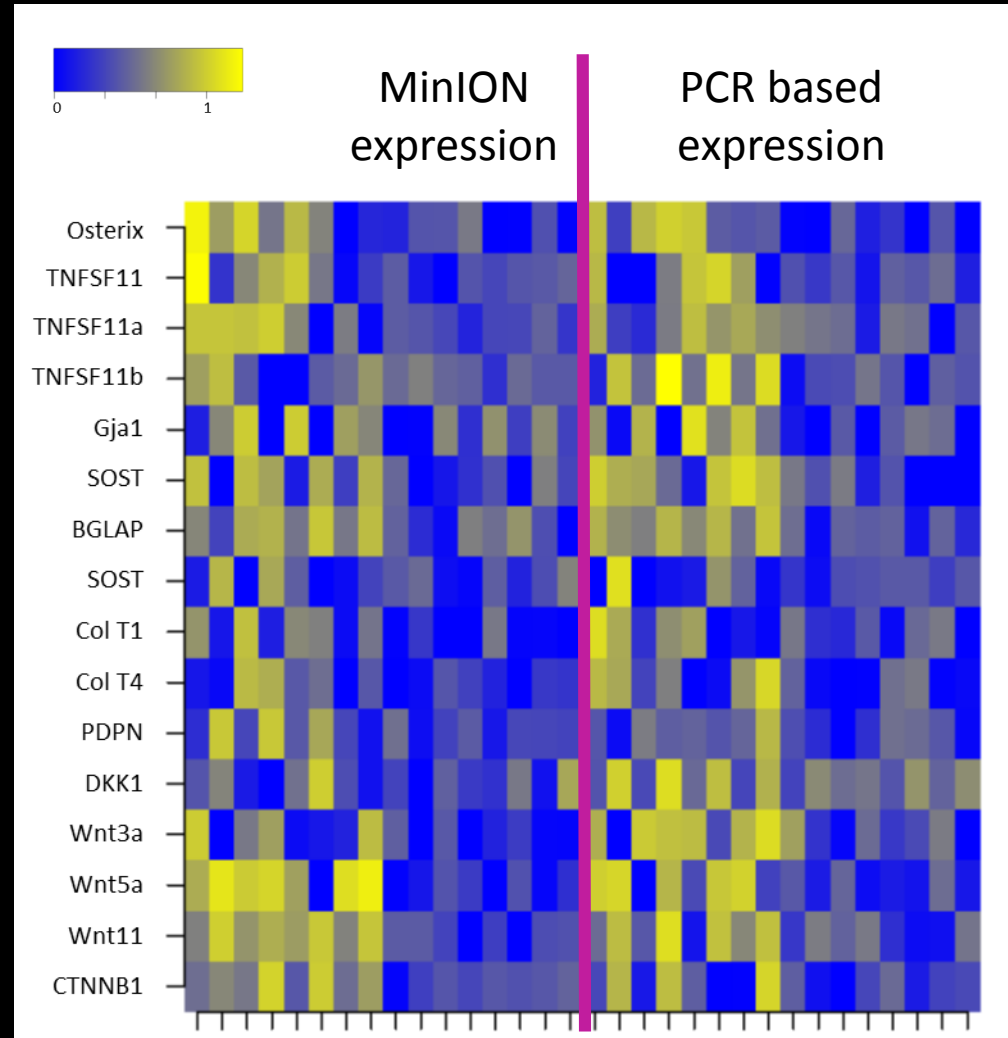


- Data is compared to a Calibration Strand for accuracy and alignment.
- 2D accuracy is above 95% for all Aligned reads.
- Average sequence length is 6,400 bases.
- Long read with high accuracy



POC: End-to-End Expression of Key Gene Sets

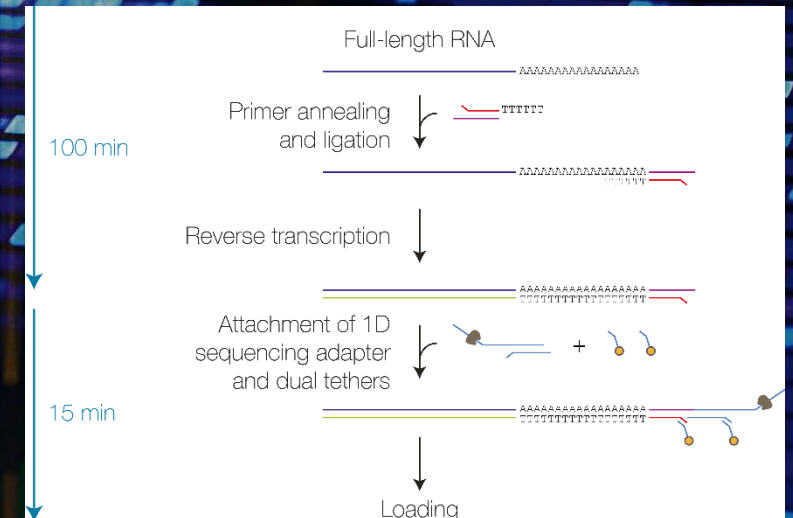
- Last using a Command Line based open source analytic tool called **poRe** visualization of expression information was established for 16 mouse genes (expression compared to GAPDH control).



Proof-of-Concept Conclusions

- We have established proof of concept for novel end-to-end molecular biology approach to sample sequencing with technology already on-board the ISS.
- Our method will enable more fundamental space bioscience investigations. Eliminate the effects of sample recovery and delay in processing.
- **Real example of continuous science in space!**

Caviat: Oxford Nanopore has greatly improved the chemistry supporting library preparation and there are now kits available which accomplish similar outcomes to those presented herein.





Acknowledgements

Eduardo Almeida, Ph.D.

Elizabeth Blaber, Ph.D.

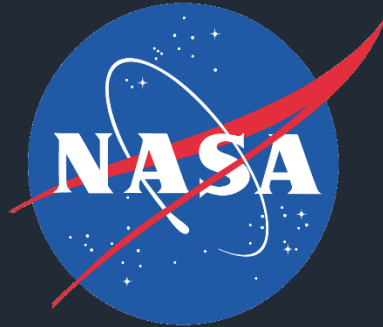
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Margareth Cheng-Campbell

Olivia Stimpel

Kristin Ma

Luan Tran (WL2 team)



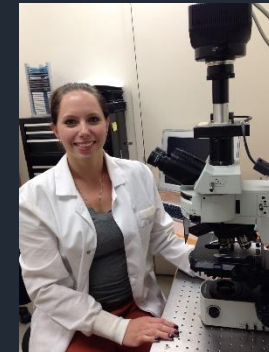
NASA AMES Office of the Chief Scientists

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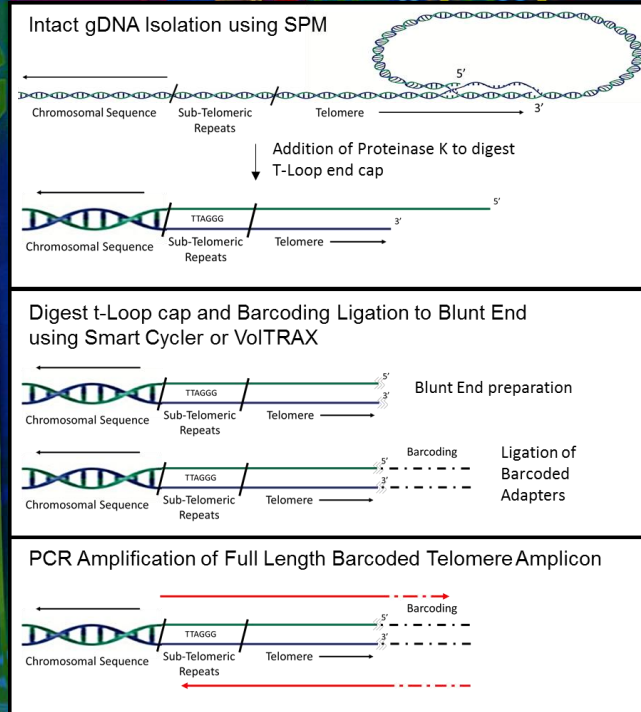
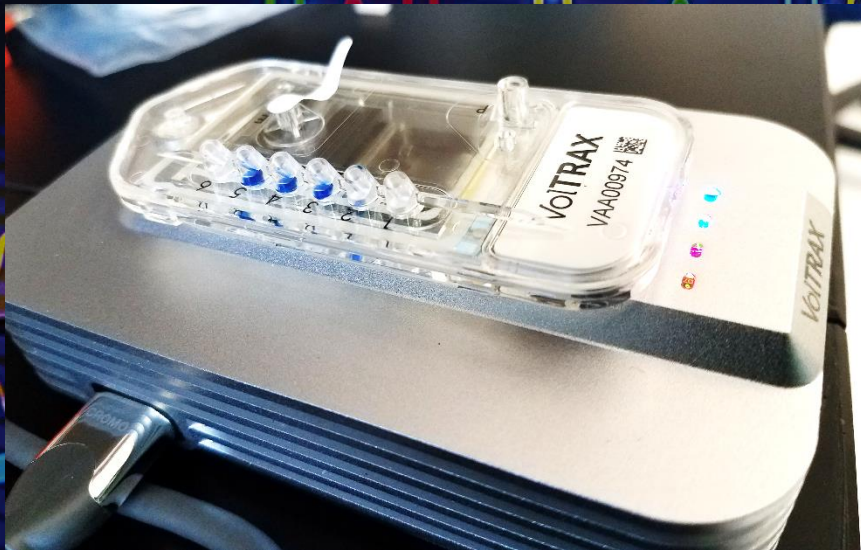
E. Blaber and NNH14ZTT001N-0063 to E. Almeida

NASA Postdoctoral Program administered by USRA

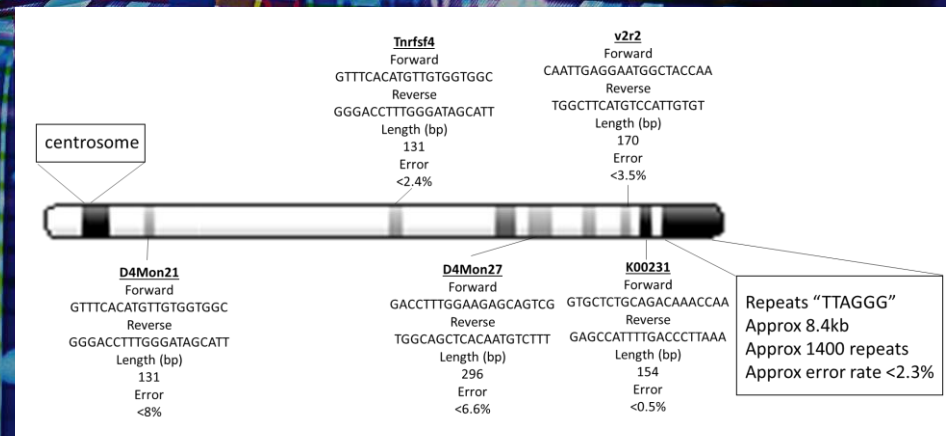


Next steps: Automation and practical application

VolTRAX automated library preparation device. Uses magnetic ferrofluids to mix and thermal cycle reagents and prepare the sequencing library



Quantification of individual chromosome telomere length at single base-pair resolution and development of a health metric to monitor astronaut general health in real time.



SP-65